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Sex in dioecious plants

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(WITH PLATES 12-14)

Probably no other question has received such universal attention among biologists as that of heredity, and perhaps no avenue of approach in solving this question promises at present greater results than that of the determination of sex. It has been generally believed until within the last few years that sex was largely determined by surrounding conditions, such as food, heat, light, or other external factors. This belief was strengthened by such work as that of Prantl ('81) on the ferns. When prothallia were cultivated with abundant nutriment only archegonia developed, whereas antheridia were formed on those poorly nourished. It should be noted however, that the prothallia with which he worked were not strictly dioecious, but had a "tendency to dioeciousness."

Similar experiments were performed upon other groups of plants, as well as upon animals, with the same general results. There can be little doubt that environmental factors do influence either directly or indirectly the development of sexual organs in plants where both tendencies exist. But recent and more careful experiments have shown that in strictly dioecious forms such factors do not determine, at least in the life history of the individual, which sex shall develop.

Strasburger ('00) working with plants and Cuenot ('99) with animals, have carefully reviewed the evidence and in their own experiments have shown that in dioecious forms sex is not determined by environmental factors but is inherent in the germ cells.

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In view of this belief regarding the determination of sex, attention has been directed in the past few years to the study of these cells. Probably no single work has done more to throw light upon this interesting question than the carefully conducted investigations on the dioecious mosses by the Marchals ('06). In experiments with *Barbula unguiculata* Hedw., *Bryum argenteum* L., and *Ceratodon purpureus* Brid., they have shown that two kinds of spores exist in every capsule in equal numbers, half of the spores giving rise to male gametophytes and half to female gametophytes. They selected mosses which may regenerate secondary protonemata from fragments of the leaf, stem, or rhizoid of the gametophyte, and in every case the sex character of the parent plant was faithfully transmitted to the regenerated portions. By subjecting the protonemata to varied conditions of growth, they were unable in any case to alter the sex of the individual. These careful experiments seem to confirm their conclusion that the gametophytes in these cases contained the characters of but one sex.

In a later paper ('07) they report the results obtained in researches on the sporophyte. By regenerating parts of the seta or of the walls of the capsule they obtained protonemata which were always bisexual in character, instead of being unisexual as in the case of those produced from the spores or from parts of the gametophyte plant. It is significant that in a great majority of cases these protonemata showed only male characters, a much smaller number showed the sex characters of both male and female, and a very few showed only those of the female. By further experiment with secondary and tertiary protonemata obtained from these apparent male and apparent female forms, they found in every case that both sex characters were really present, but that one set was latent.

Since the gametophyte generation has but half as many chromosomes as the sporophyte generation, they conclude that the unisexual character of the spores is due to two series of chromosomes which are separated at the time of the reduction division, or when the spores are formed, so that half the spores contain the series which can develop one sex, and half the series which can produce the other. At the time of fecundation these two series are again united, making the tissue of the sporophyte bisexual in character.

These experiments seemed to them to indicate that the protonemata which are produced by the spores or by the tissue of the gametophyte are unisexual. However, either one of two interpretations may be given to the results: (1) The protonemata which are apparently unisexual contain the characters of both sexes, but one set has become latent to the extent that it does not become active by artificially varying the conditions of growth. (2) The characters of but one sex are really present. This latter interpretation is the one given by the Marchals.

A similar case in the Bryophyta, that of *Marchantia polymorpha*, has been worked out, although with much less certainty. It is reported by Blakeslee ('06) that the sporophyte produces two kinds of spores in the same sporangium, one giving rise to male thalli, the other to female. The gametophytes appear to be unisexual, as shown by the work of Noll, in which he cultivated plants of both sexes by means of the gemmae for over thirty generations but was unable to change the sex character of the thalli by growing them under varied conditions.

Blakeslee ('06) finds a similar condition existing in *Mucor Mucedo*. He states that two kinds of spores are produced in a single sporangium, the one giving rise to unisexual male mycelium, the other to unisexual female. He finds that the zygosporangia are bisexual. Although neither in *Marchantia* nor in *Mucor* were the proportions of the two kinds of spores obtained, yet as far as the observations go they seem to indicate that the same general phenomenon obtains as was found by the Marchals in the dioecious mosses.

I have been unable to find any account of work done on the Pteridophyta that would indicate the occurrence of two kinds of spores in the same sporangium. Prantl ('81) and Duval-Jouve ('63) working on the ferns and equisetums, respectively, both state that there is a tendency to dioeciousness but that occasionally both sex organs may be found on a single prothallium; Strasburger ('00) also believes that dioeciousness has not been fully established in the ferns. Wherever dioeciousness occurs in the Pteridophyta, it is apparently associated with heterospory, and it must follow that in these cases the separation of the two tendencies occurs previous to spore formation. The difference then

in the dioeciousness of the Bryophyta and Pteridophyta would seem to be merely one of time as to the separation or dominance of one tendency, in the former appearing with the production of the spores, in the latter before spore formation.

Owing to the parasitism of the female gametophyte, the Spermatophyta do not lend themselves so readily to experimentation. However, the unique and careful work of Correns ('07) on *Bryonia* indicates that two kinds of microspores are formed in dioecious flowering plants; one through fertilization causes a staminate sporophyte to develop, the other a pistillate.

In this work Correns pollinated the pistillate flowers of the dioecious *Bryonia dioica* with pollen from the monoecious *Bryonia alba*; the offspring were in the main dioecious, a few staminate flowers appearing on the same plant with the pistillate but soon dying. This showed that dioeciousness dominated monoeciousness, though not completely. Furthermore, the offspring were pistillate, no staminate plants being developed; this showed that the pistillate character of the dioecious plant dominated the staminate character of the monoecious plant. Correns concludes, therefore, that all egg cells of *Bryonia dioica* bear the same tendency, and that this tendency is to develop pistillate plants.

He next pollinated the pistillate flowers of *Bryonia dioica* with pollen from the staminate flowers of the same species, and obtained in the offspring twenty-one staminate and twenty-one pistillate. Since he used flowers from the same pistillate plant as in his first crossing, the staminate character must have been introduced by the pollen spores; and since half of the offspring were staminate, and the egg contained the pistillate tendency, it would follow that there is a difference in the pollen spores.

In the third place he pollinated the pistillate flowers of *Bryonia alba* with pollen from *Bryonia dioica* and obtained all dioecious offspring, thirty-eight staminate and thirty-eight pistillate; this further showed that dioeciousness was dominant. He concludes from this and the preceding experiment that the pollen spores of *Bryonia dioica* are of two kinds or otherwise all plants would have been pistillate as in his first experiment. These results were so unexpected, he says, that he tried them over and over with different individual plants but always with the same results. These

noteworthy investigations on dioecious flowering plants, he believes, warrant the following conclusions : (1) The egg has a sex tendency which is female. (2) In each microsporangium two kinds of pollen spores are formed in equal number ; half having the tendency to develop staminate individuals and half to develop pistillate individuals. (3) The staminate tendency dominates the pistillate tendency.

Noll ('07) in his work on the dioecious *Cannabis* has been led practically to the same conclusions as those of Correns, and independently of the latter's results. It has been known for some time from the observations of Heyer, Haberlandt, and Strasburger that in a state of nature the distribution of staminate and pistillate plants in the dioecious forms is nearly equal. Heyer found, after observations on several thousand individuals, that in *Mercurialis annua* the proportion was 106 staminate to 100 pistillate ; in the same species Strasburger found the ratio to be 103.8 staminate to 100 pistillate, and in *Melandrium album* he found 100 staminate to 128.16 pistillate. In *Cannabis*, Heyer found from 40,000 plants the proportion to be 100 to 114.93 ; Haberlandt in Austria in the same species found 100 staminate to 120.4 pistillate ; and Fisch in Erlangen found from 66,000 plants 100 staminate to 154.24 pistillate. To determine the cause of this ratio, Noll first gathered seeds from various individual plants of *Cannabis* which had been pollinated by natural agencies. On growing these seeds he found that the percentage of staminate and pistillate offspring derived from the seeds of a single plant varied materially from the normal ratio. In some extreme cases only 10 per cent. were pistillate, while in other extreme cases 90 per cent. were pistillate ; it should be stated that in general there was no such extreme variation. From this evidence he concludes that the egg does not determine the ratio, otherwise there would not be such extremes of variation.

In the second place he crossed individual pistillate plants with the pollen from a single anther, with the result that the ratio of the offspring showed a very close approximation to the normal. From a plant crossed with pollen from a single anther there were obtained 100 staminate and 117.3 pistillate, while from a plant crossed with the pollen from a single inflorescence there resulted 100 staminate and 121.6 pistillate. Noll was led to believe from these experi-

ments that the ratio of staminate and pistillate plants in the offspring is determined by the pollen spore and not by the egg. Although the conclusions seem hardly warranted from the data, yet they are in harmony with the results obtained by others.

It is the belief of the Marchals, of Correns, and of Noll, that this separation of the tendencies occurs in the formation of the spores. Although dioeciousness, as the term is usually applied, refers to the gametophyte in the Bryophyta and to the sporophyte in the Spermatophyta, yet it is very probable that the separation of the two tendencies is fundamentally the same in both groups; for in both groups two kinds of spores are produced in the same sporangium or microsporangium in equal number.

From the experiments thus far carried on in plants it would seem that possible assistance in the solution of the problem of the determination of sex in dioecious forms may be had in the study of spore formation. Much attention has been given in recent years to the study of the spores in plants and to the germ cells in animals; so that at present their general morphology is well understood. It may be regarded, at least as a working hypothesis, enforced by the classical work of Boveri on the dispermic sea-urchin egg, that each chromosome or group of chromosomes bears a definite relation to the development of particular parts of the organism. With this as a basis, the works of McClung, Stevens, Montgomery, Wilson, and others on the spermatogenesis of insects are particularly significant. It has been found in nearly one hundred species that there are two kinds of spermatozoa produced in equal number, differing in the character of the chromosome complex. In these cases there are two methods of formation of the chromosomes; most of them are formed by the segmentation of the spireme thread, but the so-called "accessory" or "idiochromosomes" (Wilson, '09a) arise generally at least from a chromatin nucleolus. The difference in the two kinds of spermatozoa formed is due to the distribution of these idiochromosomes. Regarding the determination of sex these facts are of special interest, since the fertilization of the egg with one kind of spermatozoa always produces a male individual, while fertilization with the other kind produces a female. The natural inference is that it is the idiochromosomes which have to do with the determination of sex.

Wilson ('09*a*) in his comparative review of the types of sexual differences of the chromosome groups, divides the known cases into five classes as follows :

1. "Both sexes with the same number of chromosomes, a pair of equal idiochromosomes present in both. No visible difference between the two classes of spermatozoa or between the male and female somatic groups."

2. "Both sexes and both classes of spermatozoa with the same number of chromosomes; the male with a pair of unequal idiochromosomes, half the spermatozoa receiving the large one and half the small one."

3. "The female chromosome group with one more chromosome than the male. The male with an unpaired idiochromosome and an odd spermatogonial number, half the spermatozoa receiving the idiochromosome and half being without it."

4. Female group (by inference only) with two more chromosomes than the male. In the male a pair of unequal idiochromosomes, half the spermatozoa receiving both these idiochromosomes, and hence two more than the other half."

5. "Female group with three more chromosomes than the male. Half the spermatozoa receiving three more chromosomes than the other half."

Wilson ('06) has been able to show very clearly a progressive series from forms in which there is a visible difference in number or in size of the idiochromosomes to those in which there is no visible difference in either number or size, as for example those included in his first class. He regards this class "as very important as a sort of connecting link to forms, perhaps the more usual, in which there is no visible difference."

It is generally believed that in plants the chromosomes are formed by the segmentation of the spireme thread. The careful work of Allen ('05) on *Lilium*, together with the works of others on the same genus, seems to leave no doubt that in these forms all of the chromosomes are formed in this manner. In the work of Gregoire ('04) on *Trillium*, in that of Martins-Mano ('04) on *Solanum* and *Phaseolus*, and in fact in all of the literature examined, I have found no case where any of the chromosomes are reported to be formed in any other manner. A possible



exception is that of *Spirogyra*; but because of the very small size of the chromosomes there has arisen among the workers on this genus a difference in interpretation of the observations which leaves the case at present in a very uncertain position.

It is important to note that all of the work done on the formation of the chromosomes in the plant cells has been done on hermaphrodite forms.

OBSERVATIONS ON THE POLLEN SPORE FORMATION IN *Acer*  
*Negundo*

It was my purpose in taking up this study to determine if there is a behavior of the chromosomes in dioecious plants analogous to that found in the spermatogenesis of insects, and apparently having to do with the determination of sex. After examining the anthers of several of the dioecious Angiospermae, I found in *Acer Negundo* L., a maple reported to be strictly dioecious, a nucleus with comparatively few chromosomes. Another feature which makes this plant especially favorable for study, is the successive stages of development found in each loculus of the anther, the youngest stages being at the base, the oldest at the top.

The mother cells in the early stages are differentiated from the surrounding tapetal cells in possessing a greater amount of chromatin-staining material in the form of a single, spherical nucleolus. A small amount of linin is visible with a few nodes or denser portions, but these do not take chromatin stain (FIG. 1).

The first visible change of the cell during the early growth period, is a slight increase in size of the nucleus and the appearance of a small bud-like process on the nucleolus (FIG. 2). This chromatin-staining mass gradually comes from the nucleolus in the form of a spherical body; at first it seems to be attached by a thread but soon becomes free and migrates to the nuclear wall, where it becomes connected with the linin and soon diffuses out upon it to build up the spireme thread (FIGS. 3-6). Soon after the first body is given off another one appears and behaves in the same manner as the first (FIGS. 5, 6). From the fact that these bodies gradually lose their chromatin-staining capacity and the reticulum at the same time becomes more apparent, there can be little doubt but that these are masses of chromatin which go to make up the spireme thread.

As growth continues more of these bodies come from the nucleolus, often two at a time; the exact number, however, could not be determined because those coming off first lose their identity before the later ones appear. It seems certain that these bodies are coming from the nucleolus rather than going into it, for in the same section the nuclei at the bottom of the anther, which contains the youngest stages, have bud-like processes just appearing, while farther up they are merely attached, and in the uppermost parts they have migrated to the wall of the nucleus. This very marked progressiveness in development in the same anther is of prime importance in considering the successive stages.

These chromatin bodies differ somewhat in size; and as they continue to appear the denser portions of the linin take the chromatin stain more deeply, and soon these nodes begin to disappear and the thread becomes more or less continuous (FIG. 10). Owing to the size of the thread at this stage, I could not determine whether it was split or not; it appears somewhat jagged or irregular in places, often bead-like, as frequently noted by other observers at this stage (FIGS. 10, 11).

The chromatin bodies usually cease to come off from the nucleolus at about the time when the spireme thread is well formed and takes the chromatin stain deeply. Synapsis now follows very quickly and the thread always appears to be associated with the nucleolus (FIG. 12). After this stage the thread unfolds and there appear thickenings upon it; often two threads may be seen to be parallel and sometimes united for a part of their length. The thickening thus formed becomes more condensed and finally the thread segments; successive stages show that this is the method of formation of at least some of the chromosomes (FIGS. 15-18). The details in the formation of the chromosomes could not be determined with accuracy; however, it appears from the study of many preparations in this stage, that many if not all are formed by the approximation and fusion of two threads. Eight chromosomes are formed by the segmentation of the spireme thread (FIGS. 16-20). There can be no doubt about the number thus formed; for in scores of uncut nuclei only eight could be detected during these successive stages.

Just previous to the formation of the synaptic knot, a large

body appears coming from the nucleolus (FIGS. 10-12); at first it does not stain deeply with chromatin stains, but gradually it acquires the same staining capacity as the spireme thread. This body does not become freed for some time, at least not until the chromosomes derived from the thread are well formed, nor does it appear at any time to be associated with the thread in any way. During the first stages after synapsis this large chromatin body, which may be called a chromosome, shows very clearly a splitting (FIG. 13); this splitting is more or less visible until after this chromosome is freed. Occasionally, but not often, small chromatin bodies are given off from the nucleolus at this stage, similar to those given off before synapsis and these also function as those which served to build up the spireme thread (FIG. 14). They certainly do not behave like the bivalent chromosome which remains attached for so long a time to the nucleolus.

While the eight chromosomes formed from the spireme are becoming clearly defined, the one on the nucleolus gradually comes farther out and in addition one or two others make their appearance in a similar manner (FIG. 20). Each one of the chromosomes thus formed from the nucleolus shows very clearly the bivalent character; as they become freed, however, they appear more and more condensed so the splitting cannot be seen. There seems to be some variation in the number of chromosomes which appear at any one time upon the nucleolus, sometimes two or even four may be seen; but in every case no two seemed to be freed at the same time nor do two ever arise in succession from the same place. In all five chromosomes are thus derived. To illustrate the many successive stages found in the same loculus of an anther, it may be noted that FIGURES 15, 16, 20, 21, 22, are drawings from the same anther, while FIGURES 17, 18, 19, were taken from another single anther in the same stage of development.

Soon after the fifth chromosome makes its appearance on the nucleolus, the nuclear wall breaks down and the chromosomes become arranged in the equatorial plate; in the numerous nuclei examined in this stage, hardly two polar views showed the same arrangement of the chromosomes. There is also to be seen a slight difference in size of the chromosomes but the difference is not sufficient to enable one to identify any particular individual

with certainty. During the interval from the collapse of the nuclear wall to the arrangement of the chromosomes in metaphase, the chromosomes have become more condensed (FIGS. 22, 23). At this metaphase stage one or more bodies are found in the cytoplasm, which are undoubtedly remains of the nucleolus, and which finally disappear. It is very evident that these are not chromosomes located at some distance from the others, for in subsequent stages where counts can be made only thirteen chromosomes exist; and in side view these bodies have no connection with the spindle.

Particular attention was given to the first nuclear division to determine if there is any unequal distribution of the number or in the size of the chromosomes analogous to what has been found in the spermatogenesis of insects; but all observations indicate that each chromosome splits into equal halves and each half moves to opposite poles of the spindle. Polar views of the nuclei in anaphase stages of the first division (FIGS. 24 *a* and *b*), and in metaphase of the second division (FIG. 33), both show the same number, thirteen; numerous counts were made of these stages so there can be no doubt about the equal distribution. Side views of the spindle during the metaphase and anaphase stages also show, as nearly as could be determined, that each chromosome splits into equal halves; the only irregularity noticed was that some of the chromosomes in some nuclei divide before others, but in late anaphase all seem to be in about the same plane.

As the chromosomes are gathered at the poles, they are so massed that their individuality cannot be determined; very soon, however, the nuclear membrane is formed and as it increases in size chromatin masses become readily distinguishable (FIG. 25). In each daughter nucleus there is found one large chromatin mass and a few smaller ones varying somewhat in size. Many observations seem to indicate that these smaller chromatin masses pass into one large mass in one of the daughter nuclei, while in the other daughter nucleus they unite so as to form two chromatin masses of unequal size (FIGS. 25, 26, 28). As a consequence, the two daughter nuclei present a different appearance at this stage in their reconstruction by one containing one large and one small chromatin-staining mass, while the other contains but one large

one. This seems to be a very general phenomenon. In a very few cases, however, two bodies were seen in each daughter nucleus (FIG. 27). Many successive stages were observed in the same loculus of an anther (FIGS. 25-30 were taken from the same loculus) so there can be little doubt about the general order and succession of these changes.

As a general rule the secondary chromatin body to be found in the one nucleus ultimately fuses or coalesces with the larger one so that each daughter nucleus simulates a resting condition (FIG. 29). At this stage the nuclei appear very similar to the nucleus of the mother cell, possessing the small non-staining bodies about the nuclear wall. Occasionally the secondary chromatin mass found in the one nucleus does not fuse with the larger one; this lack of fusion is probably due to the shortness of time which intervenes between the first and second divisions.

Owing to the rapidity of development and to the small size of the nuclei, the formation of the chromosomes for the second division could not be followed with the detail obtained for the first. No spireme was noticed during these stages, but in many cases the spherical chromatin masses were clearly seen coming from the nucleolus, as observed in the formation of the thread for the first division. It should be noted in cases where the secondary chromatin mass has not fused with the larger one, — as sometimes occurred in the reconstruction of one of the daughter nuclei after the first division, — that this secondary mass as well as the large one gave off spherical bodies which served to build up the spireme thread (FIG. 31). In later stages many nuclei were found that showed the bivalent chromosomes coming from the nucleolus (FIGS. 30-32), clearly indicating the same general phenomenon as occurred in the formation of the chromosomes for the first division. The number of chromosomes formed in this manner could not be determined with accuracy, but there is no reason to believe that there is a difference in behavior from the first division, where eight were formed by the segmentation of the spireme and five from the nucleolus.

In the polar view of the metaphase stage of the second division (FIG. 33), thirteen chromosomes are to be seen in each plate. Owing to the small size, the division of the chromosomes could

not be accurately determined, but in side views there appeared no irregularity in the figure which might indicate an unequal distribution; it seems probable that each chromosome divides so that each of the four daughter nuclei receives the same number.

As the nuclear membrane begins to be formed for each daughter nucleus, several masses of chromatin are visible in each; and as fusion of these masses continues it is found that the two daughter nuclei derived from one of the nuclei contain one more chromatin mass than do the other two (FIGS. 34, 35). No cell walls are formed until after the second division is completed; this enables one to identify with certainty the two nuclei derived from the same mother nucleus. Although a less number of nuclei was observed in this stage than in the reconstruction of the daughter nuclei after the first division, this phenomenon of the extra chromatin mass appears to be even more constant in the reconstruction after the second division.

As to the significance of this difference in the behavior of the chromatin in the two sets of daughter nuclei, we can only conjecture. The fact that it commonly occurs in the first division and that the two daughter nuclei hand on these respective characters to the nuclei derived from them, leads us to suspect that this difference in behavior of the chromatin is in some way connected with an essential difference in its character.

Ultimately the second chromatin body fuses with the larger one, so that in the complete resting stage all four nuclei have the same appearance (FIG. 36), resembling the resting stage of the mother-cell nucleus. The four pollen spores are formed by the simultaneous division of the cytoplasm into four parts, each containing a nucleus.

#### SUMMARY OF OBSERVATIONS

*A.* All of the chromatin is contained in the single spherical nucleolus in the resting stage. The nucleolus may not be entirely chromatin, since at the time of division fragments of it are thrown out into the cytoplasm, where they gradually disappear.

*B.* The spireme is built up by the chromatin coming out of the nucleolus in the form of several spherical bodies, which may vary somewhat in size; these become attached to the linin and diffuse out upon it. Usually all of these bodies appear before synapsis.

C. Eight chromosomes are formed by the segmentation of the spireme thread.

D. Five bivalent chromosomes come out of the nucleolus after synapsis and after the spireme has become segmented into the other eight.

E. There is no unequal distribution of the chromosomes in the first division, and probably none in the second; so that each pollen spore contains thirteen chromosomes.

F. In the reconstruction of the daughter nuclei after the first division, each nucleus contains one large chromatin mass and several smaller ones which lose their staining capacity as their chromatin passes into the large mass. A secondary chromatin mass appears in one of the two daughter nuclei; this may or may not fuse with the larger mass.

G. In the formation of the chromosomes for the second division, as nearly as can be determined, the same phenomena occur as in the first division; where two chromatin masses occur in one of the daughter nuclei of the first division, the smaller mass, as well as the large one, gives off chromatin in spherical bodies to build up the chromosomes.

H. In the reconstruction of the daughter nuclei after the second division, the two derived from one nucleus contain each one more chromatin mass than the two derived from the other. This difference in behavior of the chromatin in both the first and second division seems to indicate a difference in the nature of the chromatic substance in half of the nuclei. In the resting stage, all nuclei have the same appearance, each with a single, spherical nucleolus and several non-staining bodies around the nuclear wall.

#### GENERAL CONCLUSIONS

The storage of the chromatin in the nucleolus and its later behavior in building up the thread, as noted in *Acer Negundo*, does not appear to be a phenomenon newly observed. Wager ('04) in his work on the root-tip of *Phaseolus*, believes that the nucleolus in the species studied contains nearly all of the chromatin; and that it is transferred previous to division into the spireme thread, which then segments into chromosomes. He reviews the literature upon the subject and comes to the general conclusion that in

some species the chromatin is contained in the nucleolus and in some apparently not; where it is thus contained the chromatin seems to be transferred by the thread becoming attached to the nucleolus. Cardiff ('06) figures a bud-like process attached to the nucleolus of *Acer platanoides* and thinks that it illustrates the manner of transfer of the chromatin from the nucleolus to the spireme thread. I have observed in the pollen mother cells of *Zea Mays* these bud-like processes which I believe to function in the same way. As to the manner in which the chromatin is transferred to the thread, I find, however, no case in plants, which has been thoroughly investigated, that corresponds to the condition reported in this paper, although this method of transference may not be uncommon.

Cytologists are practically unanimous in the belief that the chromosomes in plants are formed by the segmentation of the spireme thread. The only process analogous to the formation of the five chromosomes which come out of the nucleolus in *Acer Negundo* is that found in the formation of the idiochromosomes in insects. It might be urged that the case is not analogous, because in *Acer Negundo* all of the chromatin is contained in the nucleolus and that these five chromosomes are merely delayed in their formation; nevertheless the significant fact remains that the formation of these five is different from that of the other eight in that they come from the nucleolus already formed. It is to be noted, moreover, in insects that sometime during the life history of these idiochromosomes they are contained in a chromatin nucleolus, and in many cases they are at some period associated with the true nucleolus or plasmosome. So far as the observations go, this case of *Acer Negundo* seems to correspond in a general way to those insects which Wilson includes in Class I, where the two kinds of spermatozoa contain idiochromosomes of the same number and of the same size. The evidence at present is far too meager to warrant any definite conclusion as to the nature of the five chromosomes, which are somewhat suggestive of idiochromosomes in the manner of their formation.

The common occurrence of the difference in behavior of the chromatin in the two nuclei attracted my attention from the first and led me to examine the phenomenon with a view to determine



if there was an essential difference in the character of the chromatin. I suspected at first that it might be due to an extra chromosome, but further study showed that each nucleus contained the same number. It is not impossible, however, that there may be two series of chromosomes that are inherently different in character and which are, therefore, handled differently in the reconstruction of the nuclei. On the other hand, I find Wager ('04) states that in the vegetative cells of *Phaseolus* the nucleoli originate by the fusion of the chromosomes, first into a number of small nucleolar masses and then by further fusion into the large nucleoli found in the mature cell. Some of his figures seem to indicate that the fusion of these bodies does not take place at the same time in each daughter nucleus; and from an examination of figures of other works this feature does not appear to be uncommon. In view of these facts, it is impossible to judge how much weight should be attached to this behavior of the chromatin in the spore formation of this dioecious species; but the fact that it occurs so regularly in the great majority of cases during the early stages of reconstruction, leads one to believe that it may not be without significance. Considering the experimental work of Correns, it is very tempting to conclude that this may have something to do with the determination of the two kinds of pollen spores; however, we can hardly be justified in drawing any conclusion.

Coming now to the consideration of dioeciousness, the facts point very strongly to the conclusion that in the dioecious mosses, and probably in the dioecious Bryophyta generally, there is a dominancy and a separation of the tendencies in the formation of the spores. Evidently there is no such separation in the spore formation of hermaphrodite mosses; each spore must possess both tendencies. One of the tendencies, however, must dominate the other at some time during the germination of the spore previous to the formation of the sex organs. It does not seem inconceivable that this dominancy may take place in the vegetative cell, possibly due to external factors or conditions; this seems to be evidenced by the work of Prantl, Klebs, and others on various groups. Marchals' work clearly shows that fertilization brings both tendencies together, making the sporophyte tissue bisexual in character. By means of the protonemata regenerated from this

tissue they find that one tendency may be dominant and the other latent or recessive throughout the development of the gametophyte; or in other cases they find that both tendencies may be potent so that both sex organs develop.

In the Pteridophyta the tendency to dioeciousness, as noted by Prantl and Duval-Jouve in the ferns and equisetums, may be accounted for on the grounds that a complete dominance of the two tendencies does not take place at the time of spore formation, but this dominance arises at a later period depending directly or indirectly on external conditions. In the heterosporous Pteridophyta the dominance of one tendency occurs at some time previous to the formation of the spores, so that the two kinds are found in different sporangia. The essential difference, therefore, between the dioeciousness of the Bryophyta and of the Pteridophyta is that in the former there is a dominance and separation of the tendencies in the formation of the spores, and in the latter there is no such dominance and separation attending spore formation. In the isosporous ferns this dominance occurs after the formation of the spores and in the heterosporous forms before. This interpretation seems to be in accord with the work of Morgan ('09) on the phylloxerans; he finds that there are two kinds of eggs produced, a large female-producing egg and a smaller male-producing one. These eggs develop parthenogenetically, each forming but one polar body. Both eggs previous to the formation of the polar body contain the somatic number of chromosomes, twelve; after the formation of this body, the female-producing one contains twelve, the male-producing ten. Since the size of these two kinds is evidently determined at another time than at the reduction division, it may be cited as further evidence that a dominance may occur other than at the time of spore formation.

The monoecious and hermaphrodite Spermatophyta present a somewhat different condition. It seems very evident that the sporophyte tissue is bisexual in character, but that one tendency may dominate the other. Observations show that the willows, which are usually dioecious, may occasionally develop both sex organs. Another case is that of *Melandrium*, as reported by Strasburger ('00); when the pistillate flowers are attacked by *Ustilago*, the primordia of the stamens, which otherwise do not develop, produce

the mother-cell stage in the anther. Kerner shows that all stages may be found from those forms in which both functional stamens and pistils are produced, to those where only stamens or pistils develop. In fact all kinds of combinations and stages from strictly hermaphrodite forms to strictly dioecious ones exist. All of these facts might be interpreted as pointing to the conclusion that both tendencies do exist in the sporophyte but that one may be more or less dominant. If these tendencies should be of sufficient potency, then a hermaphrodite sporophyte would result; if the potency of the two varied, then one or the other of the tendencies would appear in proportion to their potency.

Considering the case of dioecious Spermatophyta, the only evidence at hand seems to indicate that there are two kinds of pollen spores, and that the sporophyte resulting from one kind will be staminate and from the other pistillate. As to the sex tendency of the megaspore there is little evidence; Correns' work seems to indicate that it contains the tendency to develop pistillate individuals. However, the evidence at present is not sufficient to warrant any conclusion as to whether there is a dominancy or a separation of the tendencies in the formation of the megaspore.

In conclusion I may briefly state the current hypotheses regarding the determination of sex. I use the term "tendency" in the sense of "power to develop," as I have in the preceding discussions.

Correns ('07) believes that the female organism contains only the female tendency and that it is recessive to the male; while the male contains both tendencies, the male dominant and the female latent.

Punnett and Bateson's ('08) hypothesis is practically the reverse of that of Correns. They believe that the male contains only the male tendency and that it is recessive to the female; while the female contains both tendencies, the female dominant and the male recessive or latent.

Wilson ('09 b) accounts for the determination of sex on a cytological basis. He concludes that the female tendency is the result of one or more X-elements which are not found in the male; this X-element may be represented by an idiochromosome. He believes that the male contains the determining factors, and so in a general way agrees with Correns.

Castle ('09) explains sex determination as dependent upon the presence of a factor or factors which are inherited in accordance with Mendel's law. Femaleness is due to the presence of this factor or factors and maleness to its absence. The differential factor is transmitted in two ways: (1) The differential factor is doubly represented in the female, which is homozygous, *i. e.*, gives rise to similar gametes. The male, characterized by but one differential factor, is heterozygous, *i. e.*, gives rise to two gametes, one possessing and the other lacking the differential factor. Sex determination therefore rests with the male parent, for when fertilization is effected by the male gamete possessing the differential factor males result. The female is homozygous dominant, not, as Correns supposed, recessive; while the male is heterozygous dominant, pure recessives being unknown. (2) The differential factor appears in but one of the gametes, *i. e.*, the egg. Therefore sex must be determined by the female (egg) since the male does not carry the differential factor. The female is heterozygous dominant and the male is homozygous recessive; homozygous dominants are unknown.

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COLUMBIA UNIVERSITY,  
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#### Explanation of plates 12-14

All figures were made by means of a camera lucida with Spencer 1.5 mm. objective and Leitz compens. ocular 12. The original drawings were reduced one third; all figures are magnified 3,000 diameters.

#### PLATE 12

1. Mother cell in early growth period with single, spherical nucleolus and non-staining bodies on linin.
2. Mother cell showing chromatin body just appearing on the nucleolus.
3. The nucleus slightly larger, the chromatin body farther out from nucleolus.
4. The chromatin body nearly free, attached by a thread.
5. Chromatin body free, a second appearing.
6. The first chromatin body attached to the linin and beginning to lose its chromatin. The second nearly free.
7. Several chromatin bodies diffusing out on the linin, others appearing. The thread becoming more apparent.

8. A later stage showing two bodies appearing at a time.
9. The thread well formed, the last bodies becoming free from the nucleolus.
10. The thread is formed; a chromosome which stains very lightly appears coming from the nucleolus.
11. The thread begins to knot up toward one side of nucleus.
12. The contraction phase with the thread in a knot at one side of nucleus, associated with the nucleolus. The chromosome upon the nucleolus takes the chromatin stain more deeply.
13. The thread unfolding; the chromosome on the nucleolus shows its bivalent character. The cytoplasm begins to pull away from the cell wall.
14. A little later stage, in which a chromatin body is coming from the nucleolus like those in 3; this is not a very common phenomenon.
15. The threads begin to become parallel and a thickening appears preparatory to the formation of the first chromosome from the thread.

## PLATE 13

- 16-19. Successive stages of formation of the eight chromosomes from the thread. The chromosome remains attached to the nucleolus but still shows its bivalent character.
20. The eight chromosomes from the thread are well formed. One chromosome nearly freed from the nucleolus, three others appearing in succession.
21. One chromosome entirely freed from the nucleolus, a second merely attached by a thread, a third and fourth just appearing.
22. Three chromosomes freed from the nucleolus, the other two apparent at opposite ends of the nucleolus. Cytoplasm well drawn away from the cell wall.
23. Polar view of the thirteen chromosomes in metaphase, showing some difference in size of the chromosomes.
- 24, *a* and *b*. Polar views of a single nucleus in anaphase, in which each chromosome has split and each half is moving toward opposite poles.
25. Early stage of reconstruction of the daughter nuclei, showing the one large chromatin mass in each, together with several smaller ones which vary in size and staining capacity. The smaller masses seem to pour their chromatin into large ones. Remains of nucleolus may be seen in cytoplasm.
26. A later stage of reconstruction showing nearly all of the chromatin lodged in one large mass in one cell and in one large and one small in the other.

## PLATE 14

27. An uncommon stage, in which there are two chromatin bodies in each daughter nucleus. In one nucleus they appear to be of more equal size than in the other; the large mass in one appears somewhat irregular due to the fusion of the smaller masses.
28. A very common stage, in which one nucleus has the one chromatin body, the other the large and small one.
29. All of the chromatin has fused into one large mass in each nucleus. The small non-staining bodies appear around the wall of the nucleus.
30. Early stages preparatory to the second division. The bivalent chromosomes just appearing on the nucleolus.
31. Later stage showing in one of the nuclei the secondary chromatin mass giving up its chromatin in small spherical bodies to form the other chromosomes.
32. A stage just previous to the breaking down of the nuclear wall; the bivalent chromosomes clearly visible, coming from the nucleolus.

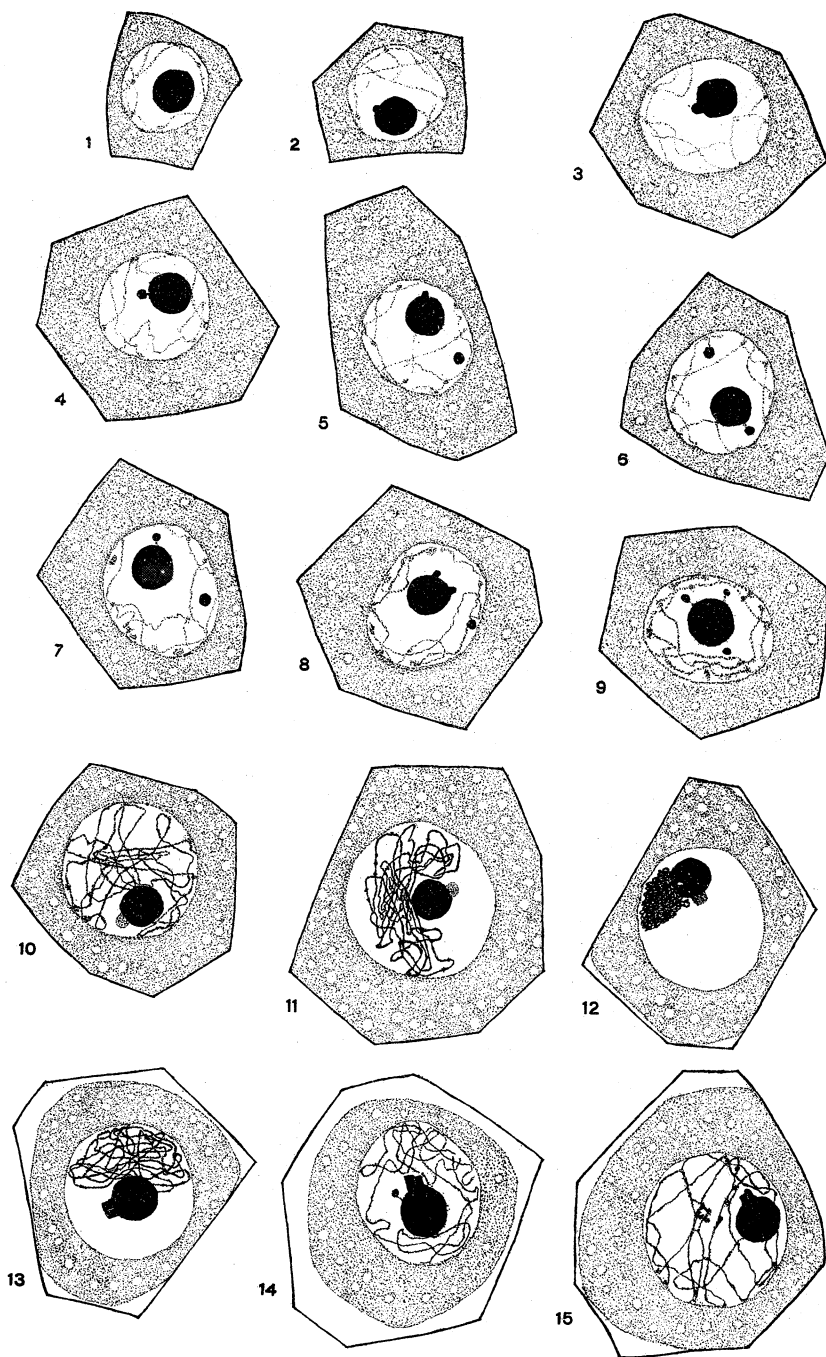
33. Polar views of the metaphase of the second division showing thirteen chromosomes in each plate.

34. Early stage in reconstruction of the daughter nuclei; the ones opposite each other are derived from the same nucleus; the two derived from one show three chromatin bodies each, the other two show but two each.

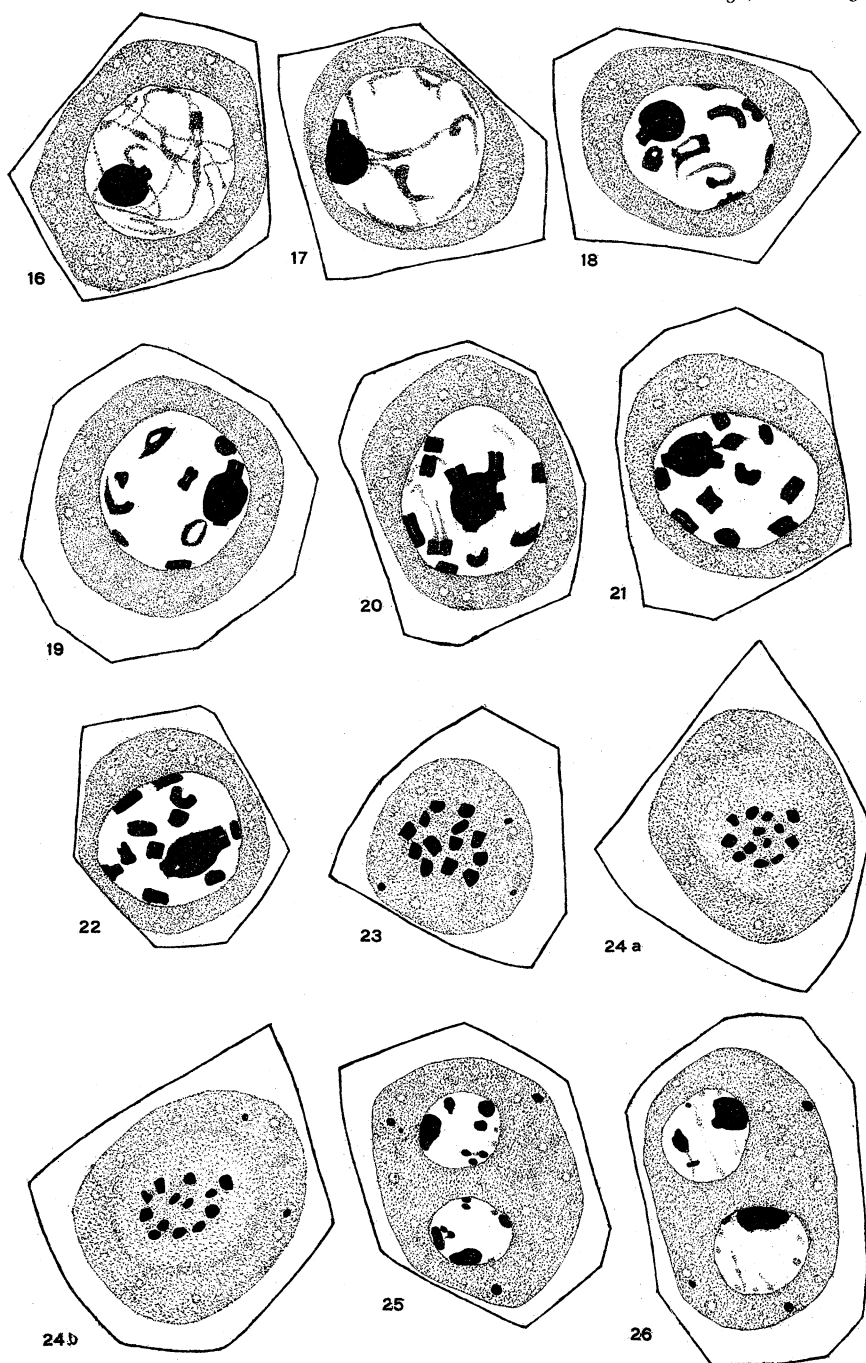
35. A later stage in reconstruction, in which the bodies are all fused into one chromatin nucleolus in two of the nuclei; while in the other two fusion is taking place in one and not in the other.

36. The resting nuclei after the second division; each nucleus contains the single spherical nucleolus with the small non-staining bodies about the nuclear wall.

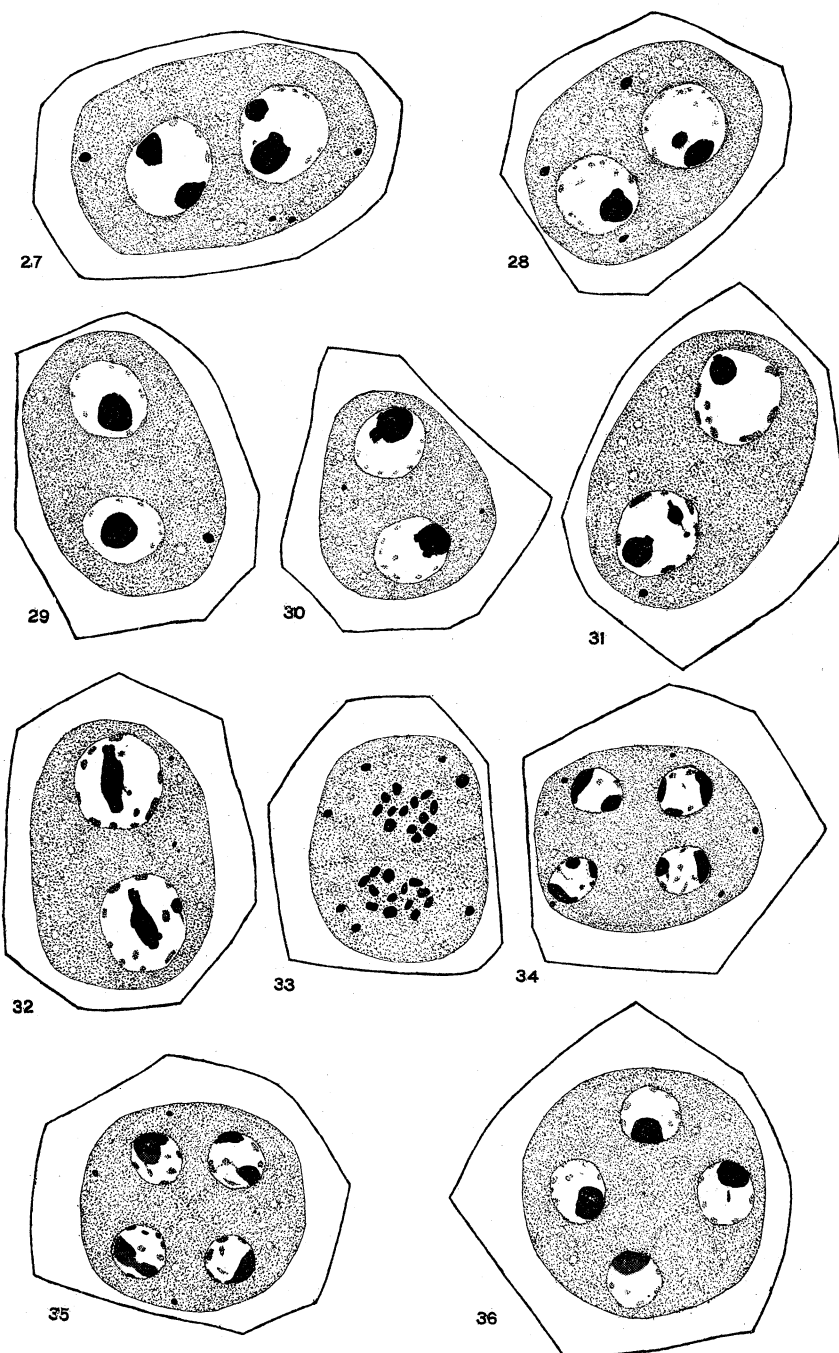




DARLING, SEX IN DIOECIOUS PLANTS



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